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PII: S0308-8146(96)00166-5

Food Chemistry, Vol. 59, No. 1, pp. 77–80, 1997

0308-8146/97 $17.00+0.00

Thermal isomerisation of β-*N*-oxalyl-L-α, β-diaminopropionic acid, the neurotoxin in *Lathyrus sativus*, during cooking

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(Received 13 October 1995; revised version received 21 February 1996; accepted 21 February 1996)

The naturally occurring β-form of *N*-oxalyldiaminopropionic acid (β-ODAP) present in *Lathyrus sativus* is the main neurotoxic principle implicated in neurolathyrism. The α-form of ODAP has been shown to be less toxic to experimental animals. Therefore, the extent of isomerisation of the toxin from the β-form to the α-form during cooking might determine the toxicity of *L. sativus* seed.

The results of the present study reveal that there is a temperature- and time-dependent isomerisation of the β-form to the α-form. The extent of conversion of β-ODAP to its α-form was determined in some common Indian cooking preparations and was found not to exceed 40%. It seems likely that the toxicity of *L. sativus* seed due to β-ODAP is only partially removed during cooking and that a significant proportion of the toxin (about 60%) remains as the toxic β-form.

INTRODUCTION

Human neurolathyrism is an upper motor neuron disease caused by consumption of the pulse *Lathyrus sativus* (Dwivedi, 1994; Spencer *et al.*, 1991; Cohn & Streifler, 1983; Haimanot *et al.*, 1990). The toxin responsible for the neurotoxicity of this pulse has been identified as the β-form of *N*-oxalyldiaminopropionic acid (β-ODAP) (Rao *et al.*, 1964). In solution β-ODAP can isomerise to α-ODAP (Bell & O'Donovan, 1966) via an unstable intermediate, 2-hydroxyimidazolidine-2,4-dicarboxylic acid (De Bruyn *et al.*, 1994). α-ODAP has been found to constitute 5% of the total toxin in seeds of *L. sativus* (Roy & Rao, 1968), and in animal studies has been shown to be less toxic. When 1-day-old chicks were injected with either 300 or 600 μg g^{-1} body weight (BW) of α-ODAP, no effect was observed, but at 300 μg g^{-1} BW of β-ODAP neurological symptoms could be observed within 30 min. Similarly, in 3-day-old mice given β-ODAP at 500 μg g^{-1} BW, severe muscle spasms and death resulted within 30 min. An equivalent dose of α-ODAP produced no symptoms and no deaths (Wu *et al.*, 1976).

A variety of animals showed neurotoxic symptoms when administered β-ODAP (Spencer *et al.*, 1986; Jahan & Ahmad, 1993; Liu *et al.*, 1989). The toxic symptoms observed in animals are different from those seen in humans, and, even among those who consume *L. sativus*, susceptibility to the toxin varies with age, sex and nutritional status (Dwivedi, 1989). Moreover, it was suggested that differences in mode of cooking can alter the toxicity of *L. sativus* seeds through isomerisation of β-ODAP to the α-isomer (Chase *et al.*, 1985). Thus there can be variability in the toxicity of foods prepared from seeds of *L. sativus*. In addition, other methods are employed to detoxify the seeds, such as overnight soaking, steeping and boiling.

The objective of the present study was to investigate the extent of isomerisation of β-ODAP to α-ODAP during cooking and also to observe the extent of loss of β-ODAP toxicity during household processing of *L. sativus* seeds.

MATERIALS AND METHODS

Analytical methods

All reagents were of analytical grade. Pure β-ODAP was obtained from Dr S. L. N. Rao, who synthesised it by the chemical method (Rao, 1975). The *o*-phthalaldehyde method (Rao, 1978) was used to quantitate the total isomers extracted in all the food samples. High-voltage electrophoresis was used to separate the isomers

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(Roy & Rao, 1968). The bands obtained were scanned using a densitometer. The combined peak areas of the α- and β-isomers were considered as 100%.

Source of samples

Seeds of *L. sativus* were obtained from the weekly local market of the village of Narayankhed, Medak District, Andhra Pradesh, India. The seeds were dehusked and split and some of them were made into a fine flour.

Extraction and concentration of samples

The total toxin in the cooked samples was extracted overnight with 70% alcohol. The supernatant was filtered off using mild suction. The filtrate of the extracted food sample was concentrated using a flash evaporator at temperatures lower than 40°C to avoid any isomerisation. This concentrated filtrate was later used to quantitate the isomers.

Processing treatment

L. sativus seeds and flour were used to prepare dishes commonly consumed by people in areas where the pulse is grown. The cooking preparation followed the methods commonly used in Indian households (Philip, 1983) and the heat treatment employed in each of the recipes is given below. 'Dal' is made from split, dehusked *L. sativus* seeds cooked in water. 'Roti' or 'chappaties' is a fried bread made of unleavened *L. sativus* flour and cooked on a hot griddle. 'Pakoda' is a fried snack, where a spoonful of batter made from *L. sativus* flour is poured into hot oil and fried until golden brown.

L. sativus flour (raw, uncooked) without any heat treatment served as a control. The controls were maintained at room temparature (28 ± 2°C). Six replicates were kept for each food preparation. Drying of the various dishes in the oven was not attempted since the high temperature involved could result in the isomerisation of β-ODAP to α-ODAP. However, roti and pakoda preparations were sun-dried and samples were defatted using hexane. Because of its watery consistency, dal was directly defatted using hexane.

Heat treatment

Standard β-ODAP solution (5.68 mM), pH 2.3, was used to observe the extent of isomerisation at different temperatures between 40°C and 100°C for 90 min. Similarly, the time course was studied at 100°C for different time periods between 30 and 120 min.

Detoxification treatment

Three methods were employed: soaking, steeping and boiling. In all the procedures, 5 g of *L. sativus* seeds (dehusked) were used. The seeds were soaked in excess water overnight or steeped with excess hot water for 2 h or boiled in water for 1 h. After the treatments, the water was discarded and the seeds were used for extraction of the toxin.

Statistical procedure

In all experiments, mean and standard deviation were calculated and the differences in mean values were tested using analysis of variance (ANOVA) and a multiple comparison *t*-test procedure. Appropriate log transformations were utilised wherever necessary for the flexible use of ANOVA. Further, correlation was done wherever required.

RESULTS AND DISCUSSION

Effect of heat treatment

The effect of temperature on the isomerisation of standard β-ODAP to α-ODAP is shown in Fig. 1. Formation of the α-isomer increased significantly ($P < 0.005$) with increase in temperature. A positive correlation ($r = 0.87$) was obtained between increase in temperature and α-isomer formation. Similarly, a decrease ($P < 0.005$) in β-isomer formation was observed with increase in temperature. Therefore, the β-ODAP and α-ODAP values are inversely related to each other. However, during heat treatment for different time periods, a greater α-isomer formation (about 40%) was seen at 90 min ($P < 0.005$); this later decreased with further increase in time (Fig. 1).

From the above observation, it is clear that the process of isomerisation is incomplete and an equilibrium is reached at the level of 60% β-isomer and 40% α-isomer with heat treatment. Earlier work (Abegaz *et al.*, 1993) showed an equilibrium between the isomers at 55°C after 20–30 h, while in the present study such an equilibrium was seen at 100°C (a common cooking temperature) after just 1.5 h. This *in vitro* observation was further extended to determine the extent of isomerisation *in situ* within the seeds.

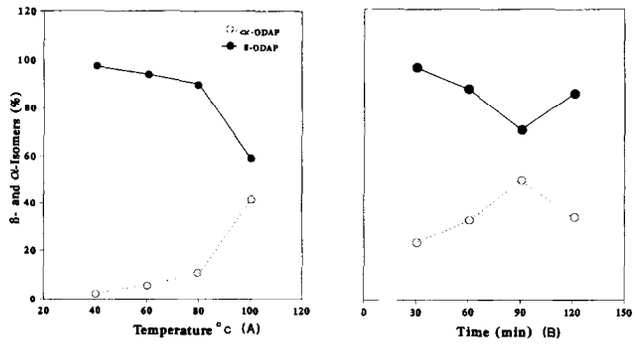

Fig. 1. Effect of temperature (A) and time course (B) of heat treatment on isomerisation of β-ODAP (standard) to α-ODAP for 90 min (A) and at 100°C (B).

Effect of processing

The influence of cooking on isomer levels of *L. sativus* seeds, when three different preparations were made, is illustrated in Fig. 2. The total ODAP content was found to be 0.64 ± 0.151 g in 100 g of the *L. sativus* seeds used. The ODAP levels in the different food preparations were as follows: dal, 0.72 ± 0.07 g per 100 g; pakoda, 0.628 ± 0.079 g per 100 g; roti, 0.624 ± 0.0204 g per 100 g. α-ODAP formation increased significantly ($P < 0.005$) during cooking. In the dal preparation made at 100°C for different time periods, a maximum of $37.5 \pm 4.96\%$ α-isomer formation was observed at 90 min of cooking. When a comparison was made between the α-isomer formation during cooking of dal and heat treatments of standard β-ODAP, no significant differences ($P < 0.005$) were observed between them (Fig. 3). In the unleavened bread (roti) preparation cooked at 140°C for 10 min, α-isomer formation of $38.4 \pm 6.10\%$ was seen. Possibly the higher temperature resulted in maximum α-isomer formation in a shorter period of time. A further increase in the time period was not attempted because of the possibility of charring the food.

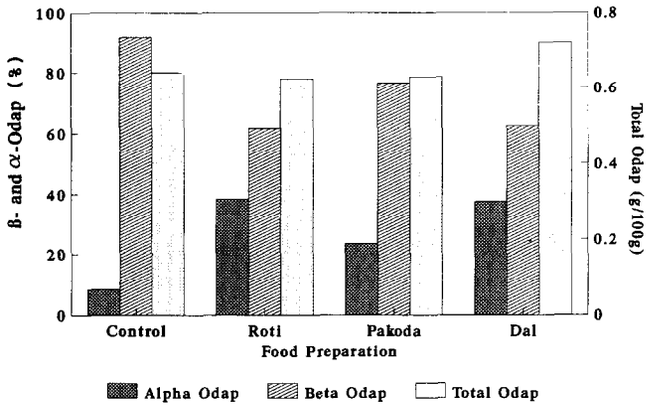

Fig. 2. Effect of cooking on the isomerisation of β-ODAP (*L. sativus* seed) to α-ODAP.

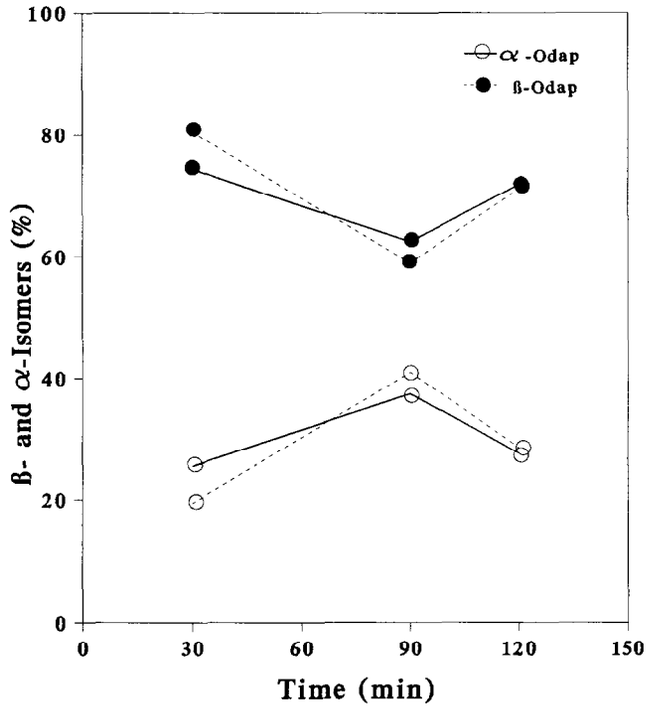

Fig. 3. Effect of heat treatment for different time periods on the isomerisation of β-ODAP (in seed and standard) to its α-isomer. · · ·, standard; ———, seed.

In the fried snack (pakoda) preparation which involved a short cooking time (5 min), $23.5 \pm 2.62\%$ α-isomer formation was observed, thus a greater amount was still present as the β-isomer. It was earlier suggested (Chase *et al.*, 1985) that conversion of β-ODAP to α-ODAP during food preparation may actually reduce the toxicity of the seed. But the present study demonstrates that the toxin is not totally isomerised and a major amount still remained as the toxic form even after cooking. This may be the reason why people who consumed cooked *L. sativus* seeds in India, Bangladesh and Ethiopia were affected with neurolathyrism (Spencer, 1989). Among the detoxification methods used to reduce β-ODAP toxicity in *L. sativus* seed, steeping and boiling in hot water was found to be more effective, by reducing the toxin content to about 90% (Fig. 4), than overnight soaking where only 50% of the toxin was removed. Earlier work also showed that, during household processing, the β-ODAP content in *L. sativus* seeds was greatly reduced only in those preparations which involved overnight soaking and decanting the water (Radha *et al.*, 1989). Further cooking of *L. sativus* seeds that had been pre-treated in salt solution and wood ash extract reduced the toxin content to about 50%, when the above solution was drained after 12 h (Urga *et al.*, 1994).

In conclusion, the present study shows that the process of isomerisation is incomplete during cooking and an equilibrium is reached between the isomers at 60% of the β-form and 40% of the α-form. A similar

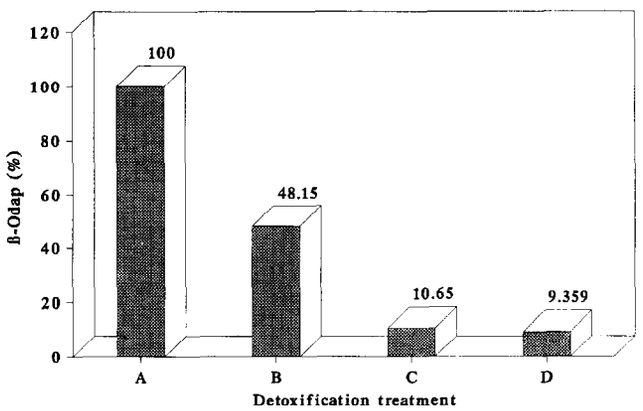

Fig. 4. Effect of detoxification treatment on β-ODAP content in seeds of *L. sativus*. A, control; B, soaking; C, steeping; D, boiling.

situation was also observed during heat treatment of standard β-ODAP solution. However, the toxicity of *L. sativus* seeds due to β-ODAP seems to be only partially removed during cooking and a significant proportion of the toxin (about 60%) still remains as the toxic β-form.

The reduction in β-ODAP toxicity using detoxification treatments was found to be a more effective and simple way of reducing seed toxicity than the isomerisation of β-ODAP to the α-isomer as observed during cooking.

ACKNOWLEDGEMENTS

We thank Dr M. Mohan Ram, Director, National Institute of Nutrition, for his cooperation and for providing facilities in doing this research work. V.P. thanks the University Grants Commission (UGC) for a Senior Research Fellowship.

REFERENCES

Abegaz, B. M., Nunn, P. B., De Bruyn, A. & Lambein, F. (1993). Thermal isomerisation of *N*-oxalyl derivatives of diamino acids. *Phytochemistry*, **33**, 1121–1123.

Bell, E. A. & O'Donovan, J. P. (1966). The isolation of α- and γ-oxalyl derivatives of α,γ-diaminobutyric acid from seeds of *Lathyrus latifolius*, and the detection of the α-oxalyl isomer of the neurotoxin α-amino-β-oxalyl-aminopropionic acid which occurs together with the neurotoxin in this and other species. *Phytochemistry*, **5**, 1211–1219.

Chase, R. A., Pearson, S., Nunn, P. B. & Lantos, P. L. (1985). Comparative toxicities of α- and β-*N*-oxalyl-L-α,β-diaminopropionic acid to rat spinal cord. *Neurosci. Lett.*, **55**, 89–94.

Cohn, D. F. & Streifler, M. (1983). Intoxication by the chickling pea (*Lathyrus sativus*): nervous system and skeletal findings. *Arch. Toxicol. Suppl.*, **6**, 190–193.

De Bruyn, A., Becu, C., Lambein, F., Kebede, N., Abegaz, B. & Nunn, P. B. (1994). The mechanism of the rearrangement of the neurotoxin β-ODAP to α-ODAP. *Phytochemistry*, **36**, 85–89.

Dwivedi, M. P. (1989). Epidemiological aspects of lathyrism in India—a changing scenario. In *The Grass Pea—Threat and Promise*, ed. P. S. Spencer. Third World Medical Research Foundation, New York, pp. 1–26.

Dwivedi, M. P. (1994). Lathyrism—a historical review. *NFI Bull.*, **15**, 6–8.

Haimanot, R. T., Kidane, Y., Wuhib, E., Kalissa, A., Alemu, T., Zein, Z. A. & Spencer, P. S. (1990). Lathyrism in rural northwestern Ethiopia: a highly prevalent neurotoxic disorder. *Int. J. Epidemiol.*, **19**, 664–672.

Jahan, K. & Ahmad, K. (1993). Studies on neurolathyrism. *Environ. Res.*, **60**, 259–266.

Liu, X., Zhang, G., Li, Y., Wang, J. & Liang, Z. (1989). Toxicologic study on grass peavine (*L. sativus*) and its toxicocomponent BOAA. *Sci. Agric. Sin.*, **22**, 86–93.

Philip, T. E. (1983). *Modern Cookery for Teaching and Trade*, Vol. 1. Orient Longman, Hyderabad, India.

Radha, A., Rao, B. S. N. & Roy, D. N. (1989). Lectins, trypsin inhibitors, BOAA and tannins in legumes and cereals and the effects of processing. *Food Chem.*, **34**, 229–238.

Rao, S. L. N. (1975). Chemical synthesis of N^{β}-oxalyl-L-α,β-diaminopropionic acid and optical specificity in its neurotoxic action. *Biochemistry*, **14**, 5218–5221.

Rao, S. L. N. (1978). A sensitive and specific colorimetric method for the determination of α,β-diaminopropionic acid and the *Lathyrus sativus* neurotoxin. *Anal. Biochem.*, **86**, 386–395.

Rao, S. L. N., Adiga, P. R. & Sarma, P. S. (1964). Isolation and characterisation of beta-oxalyl-L-α,β-diaminopropionic acid: a neurotoxin from the seeds of *Lathyrus sativus*. *Biochemistry*, **3**, 432–436.

Roy, D. N. & Rao, B. S. N. (1968). Distribution of α- and β-isomers of *N*-oxalyl-α,β-diaminopropionic acid in some Indian varieties of *L. sativus*. *Curr. Sci.*, **37**, 395–396.

Spencer, P. S., ed. (1989). *The Grass Pea—Threat and Promise*. Third World Medical Research Foundation, New York.

Spencer, P. S., Roy, D. N., Ludolph, A., Hugon, J., Dwivedi, M. P. & Schaumburg, H. H. (1986). Lathyrism: evidence for role of the neuroexcitatory amino acid beta-*N*-oxalylamino-L-alanine. *Lancet*, **2**, 1066–1067.

Spencer, P. S., Allen, C. N., Kisby, G. E., Ludolph, A. C., Ross, S. M. & Roy, D. N. (1991). Lathyrism and Western Pacific amyotrophic lateral sclerosis: etiology of short and long latency motor system disorders. *Adv. Neurol.*, **56**, 287–299.

Urga, K., Alemu, F. & Tsadik, M. G. (1994). Influence of processing methods on cooking time and nutritional quality of grass pea. In *Nutrition, Neurotoxins and Lathyrism: The Odap Challenge*, eds B. M. Abegaz, R. T. Haimanot, V. S. Palmer & P. S. Spencer. Third World Medical Research Foundation, New York, pp. 105–118.

Wu, G., Bowlus, S. B., Kim, K. S. & Haskell, B. E. (1976). L-2-Oxalylamino-3-aminopropionic acid, an isomer of *Lathyrus sativus* neurotoxin. *Phytochemistry*, **15**, 1257–1259.